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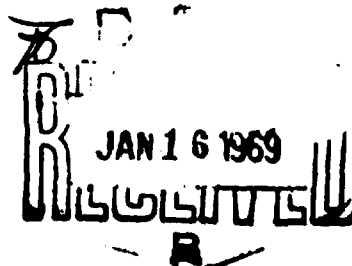
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PSITTACOSIS AND ORNITHOSIS

by Jean Viouchango

Head of the Laboratory Institute of Pasteur, Paris
(Virus Service)

In the case of parrots, psittacosis is present in animals as generalized infection with a predominance of digestive symptoms. Transmissable to man it takes on the characters of an infection of a typhoid type, during which in general pulmonary complications set in. One always finds at the beginning of human epidemics a contact with sick birds. It was essentially this epidemiological character which allowed the clinical identification of the disease.

The experimental research taken up as a consequence of the great pandemic of 1929-1930 resulted in a complete revision of our knowledge of etiology of psittacosis . They demonstrated that the agent of this disease was an ultravirus.

Chapter I

History

In 1879, Ritter observed at Ulster, in Switzerland, seven cases in humans of pneumonia infections and carrying the diagnostic features of pneumonic typhus but he noted at that time there was neither pneumonia nor typhus in the regions. The development of this epidemic coincided with the importation of parrakeets. These animals showed certain signs of sickness after which one of them died. Ritter (1) established a relationship between the development of a human epidemic and the aviary epidemic. He sacrificed animals which had survived: Earlier the bacteriological examination of the organs made by Eberth did not result in positive results.

Three years later facts were observed at Berne. A few cases were noted at Leipzig in 1886 and in 1887 at Bonn.

Following the importation of parakeets that in 1892, an important epidemic sprung up in Paris. In December 1891 two merchants brought five hundred parakeets from Buenos-Ayres. Half of these birds died on the trip. On their arrival in Paris on the 3rd of February 1892 there were only about two hundred parakeets left. They were separated into two lots which constituted the two principal foci of the epidemic. The secondary focus was determined by the sale of infected parakeets. The first focus originated in 22 human cases among which there were six deaths; the second there were 20 cases and 8 deaths. For Nocard (1) it was firstly purely an epidemic of infectious pneumonia which in no way resulted from the disease of parrots transmitted to man. In the report to the Council of Public Hygiene, Dujardin-Beaumetz (2) considered the symptoms shown by the patients were of an infectious grippe type, and despite the fact that the parakeets seemed to have been the cause of the epidemic this was only an appearance, in reality the epidemic had spread from man to man; he cited a certain number of cases in patients who had never come in contact with the responsible parakeets. In considering these facts Peter (3) presented the hypothesis which was

a case of typhus epidemics with relapses, spontaneously created by parakeets which had been subjected to generally unhealthy conditions— a faulty interpretation which underlines the aviary origin.

A second epidemic similar to the first appeared the next year in January 1893. An investigation conducted by Dubies (4), lead him to discard the idea fof a common infectious pneumonia. He adopted the idea of a special infectious illness, determined by contact with infected parakeets and shows that the pneumonia manifests itself "epiphenomenon, a acute additional complication nearly always rather tardy".

Dujardin-Beaumetz (5) then retracted his original opinion and admits that the sick parakeet might be the factor of inffection.

The hypothesis of a special disease was/by the discovery made by Nocard (6) made in 1892 of a special microbe found in the bone marrow of infected parakeets. It was a negative gram bacillus, short and very mobile. The bacteriological examination was concerned with the dried up wings of the parakeets which had died during Buenos-Ayres trip approximately four months earlier. This germ was found in all the examined wings. It could be cultivated. Pathogenic for parrots, the pigeon, mice, rabbit and also in a lesser degree the guinea pig and the dog. The experimentally started disease in parrots presented the same characteristics of the spontaneous illness. Nocard concluded that the epizootic. and the related human cases were caused by this microorganism.

Three years later in 1896 Gilbert and Fournier(1) during a new small epidemic confirmed the work done by Nocard in what concerns the presence of this bacillus in the blood, the viscere, the intestines, the bone marrow of the diseased parrots. However they were unable to find it in man during the illness. Only in one case were they able to isolate it in the blood taken from the heart of a person who had died of psittacosis (2) . They also showed that agglutination reactions can be obtained by having the serum of the disease act on the bacillus of Nocard. However,later, with new patients, these very same authors (3) were unable to bring the agglutinating power of the serum into evidence. Sicard (4) and Nicolle (5) also obtained only negative results both in the agglutinating powers of the Nocard bacillus in material of human origin.

While the epidemic foci had nearly completely vanished in France new ones appeared in 1897 in Italy and, under the same conditions following the importation of parakeets. An acute epidemic appeared in Germany in 1899, during which Leichtenstern and Czaplewski (6) invalidated Nocard results and only attached the value of the hypothesis of the relation between and the human epidemic. During the following years small isolated foci were signaled in various countries: In Brasil (7) and in the state of New Hampshire, US, in 1904 (8) in England1914 (9) in Pennsylvaina, Unites States, in 1917, and again in Great Britain 1924 (Edinburg), in 1926 (Birmingham) and in 1927 (London). These epidemics not numbrous and not important remain localized. A contrast with the great psittacosis endemic of 1929-1930, during which one was able to notice very numerous foci in most of the American and European countries.

It started during July 1929 at Cordoba (Argentine). After the manifestation of the first cases the doctors diagnosed it as the Grippe. One of them Barros diagnosed it as psittacosis (10) and (11). When the true character of illness was made known the country's bird merchants rapidly sold their stock to foreigners at very low prices. The importation of those animals which was at the origin of the disease

which appeared in Europe and North America. The epidemic was especially acute in
Germany, in England and in the Unites States. The sanitary statistics give the
following numbers: Germany, 215 cases (45 fatal); Unites States 169 cases (33 fatal);
England 125 cases; France: 7 in Paris and 13 at the Havre; Austria 7;
Switzerland 5; Checheslovakia 6; Holland 9; Denmark 5; Sweden 7; Poland 2; Italy 5;
Algeria 9; Canada 9. A certain number of cases were also mentioned in Spain,
Portugal, Cuba, San Salvador, Guatemala, and the Hawaiian Islands.

Because of the importance and of the wide spread nature of this epidemic
it caused numerous investigations which had its goal in particular to define the
diseases' etiology. Bedson and his collaborators (1) first demonstrated
that the blood of the patients sterile in the usual culture media, inoculated by
intraperitoneal or even intramuscular means to zebra parrakeets, was able to communicate
a disease transmissable in series to these birds. They were able to complete
the same demonstration with material filtered and non-filtered coming from diseased
parrots.

Shortly after the publication of the two British authors, a note by Levinthal
(2) appeared in Germany which described very small corpuscles found in the residual
of organs or organic liquids and to which the author contributed an
etiological significance. The discoveries made by Bedson and Levinthal were soon
confirmed on all sides; by Krumwiede (3) and his collaborators in the US who
also were able to transfer the disease to the white mouse; in France, by Secquepee
and Ferrabouc (4). On the other hand Coles (5) and Lillie (6) rediscovered the
corpuscles described by Levinthal.

During the years which followed this worldwide epidemic, new cases of psittacosis
have been noted. However, in general, the source of infection was a bird coming
from the local source and not imported.

Thus in the Unites States, psittacosis was noted at the beginning of 1931 at
Brooklyn and during the last three months in New York and California (7). The next
year 76 cases with 7 deaths were noted; and in 1933, fifteen of which 4 were fatal (8).
In 1934 an epidemic described from Pittsburgh during February and March, during
which 37 people were affected.

Two cases were noted in England in 1932 and 3 in 1933 (1). In the Netherlands,
6 were recognized at the end of 1933 and the beginning of 1934 (2). In Germany
a few cases were noted at the end of 1931 (3); in 1933 and 1934 seventeen small
epidemics were apparent; 32 cases were reported (4). In France, isolated foci
were manifest in 1932 (5) and in 1933 and 1934 (6, 7 & 8).

In June 1934 following these epidemics, the permanent committee of the Office
of International Public Hygiene (9) published a report on the measures
which should be taken in a double way for both prophylaxis and to diagnose
the sickness.

No epidemic has taken place since that date; however small foci spring up from
time to time in regions more or less distant (such as that studied by Thalheimer (10)
in Paris in 1937). They were proof of the always dangerous possibility of a reoccurence
of the epidemic and of the necessity to maintain extremely strict prophylactic measures.

We will later see in the chapter consecrated to epidemiology, that the psittacosis virus is not only found among psittacides; numerous other birds might harborate: canaries. It was also discovered that a virus not quite identical to that of psittacosis however very close and called ornithosis virus was a cause of pigeon disease and disease among domestic hens, it was there fore labeled ornithosis.

The systematic study of the characters of virus of psittacosis, ornithosis have allowed to bring them closer to other virus, one known earlier that of venerio lympho-granulomotosis (the diseases of Nicolas and Favre), or more recently identified such as atypical pneumonia, meningopneumonia, and cat pneumonia. These studies have resulted in elaboration of a classification of the k whole which allows one to arrange these various virus in one same group and also to arrange special methods of idenficiation.

CHAPTER II

Clinical Characteristics

I. Human Disease

The clinical picture of psittacosis has principally been designed by French authors as a consequence of the 1892 and 1893 epidemics. As we have seen the merit of having considered this disease as a quite separate illness goes to Dubief, until then it had been considered pneumo-typhus (Ritter), the grippe (Dujardin-Beaumetz) or for reccuring typhus.

The description as given at that time and during the next few years by Dubief,(1) Gilbert and Fournier (2), Dupuy (3), was found entirely confirmed by the authors who observed the epidemic of 1929 and 1930 and particulary Adamy (4), Sturdee and Scott (5), Mayer, Gerke and Geist (6).

The average/duration of the period of incubation can be six to approximately 8-10 days (Gilbert and Fournier, Elkeles (7), Enbden and Adamy (8), Hordeschee (9), Kerschen-steiner (10). Shorter (4 days: case of Hegler (11) or longer(21 days) delayes have been noted. Levy Simpson estimated in general the period lasts approximately 15 days, but may be shorteded to 10 days or lenghtened up to 18 days at the most.

The signs of attack are no different from the great typhoid infection; the illness starts with a general feeling of general pains, often intense headaches, rachialgy, nausea. Temperature rapidly reaches 39° to 40°. Epistaxis, diarrhea, vomiting have been noticed in certain cases. The patients sometimes present a periticcal, more rarely stomaTiTis or angina. These signs are usually entrenched within a few hours. However, cases have been noted where the disease picture comes slowly, progressively, in four to five days.

The period of state is characterized most of the time by exhaustion and extreme prostration of the patients (Dubief) accompanied by sub-delir an or continued delirium (Gilbert and Fournier). During the entire crisis period the temperature froziins at approximately 40 to 41° without very heavy morning changes. The patients are often of extreme palbuy (Weltmann (1) which associates the profound intoxication of the organism. "pink blotches" have been observed from the seventh to the fourteenth day Hutchinson, Rowlands and Levy Simpson (2), in the form of the skin covering of the chest, of the abdomin and the patients back.

Thirst is great. Nausea and vomiting sometime continue. Constipation is usual. Urination is rare, dark, albuminous. Gunther (3) insists on the fact that he has never observed ·····   . During the biginning of the crisis there are no signs of particular ········ respitory signs except sometimes a sinematería polytnea (Aujlonn and Jude). However on the very day following the first symptoms one may observe pulmonary signs; they sometimes show themselves later, around the 7th day from the start (Gilbert and Fournier), or even towards the 11th day. The cough usually comes first, but might be missing. Certain authors consider it exceptional (Gunthur). The disease presents abundant expectoration; somtimes this is lacking. During a somatic examination one finds foci of pulmonary congestion of a transient type, developing by successive thrusts, evolving towards hepatization.

The novel disorders ········   normal concentration. However, the pulmonary phenomenon continues to dominate the scene because of their intensity to appear.

Thus the infection may be attributed to a reaction on the heart. The fever rises until it reaches 41°. The patient is in a perfect typhus stage, with patpho circulology and tenderness jumps. Dyspnea becomes extreme and final coja sets in. Death follows in the 2nd or 3rd septenoria. In casese evolving towards recovery, the symptoms usually decrease and disappear between the 8th and the 10th day. The fever returns to normalafter gradual decline and lysis. the average total duration of the disease is 3 weeks. The convalescence is always very lengthy.

In addition to the intense and acute typcs, all the others have observed attenuated forms, benign, in which the fever does not go over 38 or 39 degrees with Gonerally light signs, without pulmonary affliction. The evolution of these forms is short and does not last more than 8 to 10 days. They are mostly observed among children and young patients.

The prognostic of intense types is always serious. Old Age, former organic damages lesions are the main factors in the acuteness of the disease. However it is impossible to prognosticin the evolution of this disease beyond these notions. Levy Simpson however consider that a number of pulsations below 100 is a favorable indication.

Mortality is high. Villienne (1) LISTS counts 22 out of 55 patients, ie 37, 28 %. According to Dupuy, this % was 44, 28 from 1892 to 1897. During the pandemic of 1929-30 mortality was below 20% ············ among a limited series observed by Levy Simpson. Weltmann values it-at-45 % ESTIMATES IT T. CE 45 %

II. Psittacides natural disease

The period of incubation among receptive birds is variable. It switches from 2 to 5 days or is prolonged into a few weeks.
MOSTLY MAINLY
The disease is/manifested by general and digestive signs. The animal becomes prostrate, sleepy, immobile, refuses to eat. The feathers stickup, the wings droop. The bird soils his cage and feathers with an abundant green o'fensive, often bloody diarrhea. In general, one observes an ocular nasal catarrh. Loss of weight becomes more pronounced and death follows in approximately 8 to 9 days. However all the cases are not fatal; there are many benign and abortive cases. If the animal does not die at this time the diarrhea stops and appetite increases. Respiratory difficulies might appear during the evolution; dyspnea with beating of the wings, coughs; but usually they remain in the background.

The chronic cases must also be underlined as they are extremely important from a epidemiological point of view in the spread of the illness.

Chapter III
Epidemiology

During the 1892-3 epidemic and the pandemic of 1929-30, the first human cases were in contact with imported sick parakeets or parrots. The secondary foci appeared in places where animals from the original lot had been repartitioned. On the other hand, in the following years, the source of infection, was in nearly all cases, not from imported parrots, but from animals raised in the country where the epidemic had manifested itself.

The development of these beginnings is explained by the existance of virus carriers, these being both cured animals, or health carriers. Meyer and Eddie(1) have as a matter of fact noted in California husbandries that latent infections are more frequent than apparent disease. This fact has been demonstrated in other forms, in South America and Australia.

The beginning of infection in exported animals is due to bad transport conditions, to changing of climate, and to the number of birds brought into-contact. ......

In cases of contagion from sick birds, the disease if often directly transmitted, both through contact or through bites; there are many cases of this type of contagion among proprietors who feed their parrots from mouth to mouth. The contagion can also come about indirectly, through nasal secretions and the spittles which parrots have the habit of throwing around them, or from their defector, urines and feces. This type of indirect transmission has been noticed among subjects who had simply cleaned the cages of sick birds or manipulated the carcasses of infected animals.

On the other hand it is certain that psittacosis may be given to man through the intermediary of apparently healthy birds, virus carriers. Hegler(2) speaks of a small epidemic which had a healthy parrot as a cause. Lons and Kreuche(3) relate the case of a parrot who had been living in a home for a year and suddenly started an epidemic.

However we have to insist on the fact that parrots and parakeets are not the only factors in transmitting psittacosis. Hoge(1) states that all the birds of the psittacide family may be actual or potential carriers of psittacosis; amazons, double headed mexicans, grey african parrots, cockatews, aras, loris, inseparable parakeets and allsimilar birds. Meyer and Eddie even found the virus in the latent stage in the canary(2). Roucakine (3), Sturdee and Scott(4) and Armstrong(5) had already spoken of human cases in relation to canaries.

In 1940 on the one hand, Soles(6) demonstrated that the virus of psittacosis was the cause of South American pigeon disease, and on the other hand Pinkerton and Swank(7) in the United States isolated a virus morphologically identical to that of psittacosis among pigeons restricted to a diet lacking in thiamine; however, this last virus while it was able to provoke a deadly meningo encephalitis in mice through intracerebral inoculation was deprived of pathogenic power when inoculated peritoneally.

It was possible for Meyer (8,9) and Meyer, Eddie and Yamanura(10) to trace the relationship with diseased pigeons in the history of certain human psittacosis patients in the U. S.. In England, Andrewes and Mills(11) discovered the virus among apparently healthy pigeons.

These observations, confirmed by Smadel, Wall and Greg(12) and Zichis, Shaughnessy and Lake(13), led to recognition of the existence of a Psittacosis type infection

among pigeons, an infection which Meyer offered to name "Ornithosis". For, as had already been observed by Coles(14) and Pinkerton and Swank(15), the virus isolated by Meyer from pigeons is not pathogenic for mice when it is injected peritoneally, which already indicated that one was not in the presence of 2 completely identical viruses. Further on, in the chapter on experimental study, we will see a few more differences.

The disease has also been discovered among chickens: Meyer and Eddie(14). The virus isolated from sick chickens is in every way similar to that isolated in the pigeon; by intramuscular injection in the chicken, it produced a fatal disease Eddie and Francis(1) showed by the study of the serum by means of the reaction of deviation of the complement, that turkeys and domestic ducks may in a large proportion carry the virus.

It appears that the sick pigeons are more dangerous than mere carriers, for they spread the virus in far greater quantities. It is enough to think of the great number of people, who in the country and in parks and public places in towns, constantly live in the proximity of chickens, ducks, turkeys and pigeons to realize the importance of the problem.

Cases of contagion from sick to healthy human beings exist. However they are very rare(Kerschensteiner, Gujthur, Aujaleu, etc,).

On the other hand, during the development of an epidemic one may observe a susceptibility of individuals which nothing may explain. On this subject there is only one precise fact, the age of the individuals; the patients, are in general, adults.

One may suppose that among men, the lack of certain assets probably favor the development of infection: infact, one noted above that Pinkerton and Schawanlt observed that faulty elementation or a lack of thiamine may start latent infections among pigeons.

Chapter IV
Experimnetal Study

We have stated, that after a description by Mocord in 1893 of a gram negative bacillus, short and very mobile, isolated from the bone marrow of parrots that had died of psittacosis, it was admitted in a general way, that this bacillus was the cause of the disease.

However this germ could never be found in a person during the period of infection The 1930 pandemic was the starting point of a series of experimental studies which permitted to discount Mocard conclusions and demonstrate that the disease was caused by an ultravirus.

Bedson, Western, and Levy Simpson, at the beginning of 1930 reported(1) that they had studied 12 human cases which clinically presented themselves of having been infected by psittacosis and 6 parrots in relation with these cases. Their bacteriological research remained negative; they were neither able to isolate a bacteria of the salmonella group bacteriologically or serologically; but by employing the zebra carrebeet as an experimental animal, they demonstated the presence of a filterable virus in the organs of the parrots and in the material derived from the human cases (citrated blood, serum, or pleural exudate). Under these diverse conditions they obtained virus strains which allowed them to transplant the disease to laboratory animals in series.

Receptive Animals.

Many animals were receptive. The pathologic aspect is extremely variable. It depends on the subject and on the means of entrance of the virus. Moreover, the strains of ornithosis are considerably different from the strains of psittacosis, in what concerns the pathogenic strength.

1. Psittacides

Research on the parrot was conducted by Rivers, Berry and Sprunt (2). It is possible to infect these birds by the digestive means, by the nasal or the intramuscular means. The disease develops in various ways after these inoculations; it might result in an acute form in a few days or in a chronic form and last a few months. The animals might die suddenly without having given an appreciable sign of disease. The general rule, however, is that they lose weight, their feathers stick up, their feces become liquid and one may observe a nasal secretion during the entire course of the disease. The majority of infected parrots died.

The disease follows the same line in the case of the parakeets. Bedson and his collaborators used the type Melopsittacus undulatus or zebras parakeets, for their first experiments in isolating the virus. The receptiveness of this is perhaps not as great as that of the parrot for Bedson discovered that the strains of virus conducted in series of the parrakeets type had a tendency to lose their virulence.

However, the psittacides may be carriers of virus without showing any sign of disease. Moreover, experimenting with these birds is dangerous because of the contamination. These difficulties have resulted in the choice of other animals.

2. The Hen.

The domestic hen has shown itself receptive (1), in a lesser degree than the parakeet. Five to nine days after inoculation the animal becomes careless, sleepy; it refuses food and stays in one corner of its cage with closed eyes with its head and tail hanging. It might stay this way for a few days and die in the state of pronounced emaciation. In certain cases, the hen apparently does not react to the inoculation, however its x liver and spleen are virulent.

3. Other receptive birds.

A canary is receptive (Elkeles (2)); Meyer and Eddie (3) have demonstrated the presence of the virus in canaries appearing healthy. Levinthal (4) had positive results in transferring the virus to the Japanese rice-bird. Also receptive are the finch, the bengali, the calfat (5).

The pigeon , greenfinch, and linotte appeared refractary (Bedson).

In connection with this it is important to remember that the pigeon (Streplo-pelia doca octo and Streplopelia semitorquata) may spontaneously be infected with the psittacosis virus originating in parrots or the parakeets when in contact with these birds in zoological gardens (Tombinson 6).Moreover,Pinkerton and Swank (7) noticed that the ornithosic virus causes/meningitis infection with regularity with the pigeon while the psittacosis virus of humans or avairy origin, experimentally injected into this type of animal, only rarely cause the aparation of infection and only in cases where viruses have recently been isolated.

4. Mice.

Krumwiede and his collaborators (1) were the first to publish on the receptivity of mice inoculated intraperitoneally with material taken from the parakeets. Moreover, they demonstrated the active agent could be transplanted from mouse to mouse. This data was confirmed and completed by Gordon (2), Rivers and Berry (3). These authors inoculated the virus intracerebrally, and were able to obtain an indefnite transmission, by inoculating from brain to brain.

In the case of intraperitonsally inoculated mice, the beginning of the illness varies with the quantity of injected virus and the virulence of the strain. The animal loses its appetite, loses wieght, its hair stands up, The lapse of time from the inoculation to the animals death varies. A great number of mice died within 48 hours others die off in three weeks. A very small number is cured.

Transmittal in series is easily obtained by intraperitoneal injection of an emulsion of liver and spleen. These transmittals in series do not diminish the germs virulence in what concerns the parakeets, on the other hand they increase it in the case of the mouse: During the first transposals the inoculations of 0, 5 cubic centimeters of an emulsion of organs of 10% kills animals in 4 to 5 days and a few mice in survives. However, after the fortieth passage all the mice die regularly in 48 hours.

Mice show no symptoms of disease the day after intracerebral inoculation and even a few days later. The first signs consist of bristling of the hairs, hyperexcitability . The animal doesn't eat anymore. It stays in a bow with an arched back and head droping to the ground between the periods of hyperexcitability. One may often observe the ataxy, the mouse turns around in circles, tries to jump and fall back. This is followed by convulsive fits which is ended. The position of dead mice is characteristic: the head is "retracted", the back arched, and front legs are bent while the back legs and tail are extended. The death date varies with a dilution with a virus used for the inoculation. A dilution of 1% kills within 40 to 60 hours; another at 0, 00001% in six to seven days.

Moreover, Rivers was able to isolate the virus, by inoculating intraperitoneally into the mouse from a human subject during tenth day of illness. He showed what use this method could be in the case of practical diagnostical disease (1). Inoculation of psittacosis virus in the mouse through the respiratory organs used in chemotherapeutic experiments by Hauer (2) was systematically studied by G. Hormus (3) (4). The mice died in two to three days following intranasal inoculation while under chloroform anesthetic. The mice are extremely during the first to sixth hour preceding their death; respiration is short; often, at the moment of the animals death, a/ quantity of bloody lathery foam appears on the lips and the nostrils. This liquid examined under the microscope, is poor in cellular elements and contains and abundance of elementary typical corpuscles.

When the virus is diluted, a lessening of the virulence by nasal inoculation results, which can be compared to that observed in peritoneal inoculation cases.

Passage from lung to lung does not seem to modify the virus: the experimental disease involves in the same way and in the same details; or completely different. As a matter of fact, inoculated cerebrally, they determined that the appearance of a meningo-encephalitis which is deadly, and in every way compares with the psittacosis virus injected under the same circumstances.

Elementary bodies morphologically identical may be observed on the smears: Either independent or in intracellular groups. On the other hand, injected intraperitoneally the virus of ornithosis has no pathogenico power whatsoever as observed by Pinkerton and Moragues (5), or a very feeble one as noted by Coles (6) and Meyer, Eddie and Yanamura (7).

5. The Guinea Pigs.

The guinea pig is much less receptive than mice. Intraperitoneal inoculation only allows one to observe a thermic elevation. (Bedson and Western 8). However this fever reaction ....,
Temperature becomes normal after three days. Examination of the liver and the spleen to study the virulence, shows that the virus has multiplied, however without obtaining the degree of development which one has noted in the mouse. ....
.... for this animal: after the third passage, the virus is generally lost (Bedson 1).

The intracutaneous inoculation produces up to 2 to 3 days a pimple of which resembles the type of reaction produced by the herpetic virus: there is not formation of vesicules. On the sole of the paws one may/noto redness and swelling, lesions which reach their height on the third or fourth day. Passage of this material to the mouse prove that it is an in situ virus pullulation. Bedson and Western utilized this reaction of the skin of the guinea pig as a method of virus standardization.

They later were able to obtain a lesion transmissable in series from the guinea pig, by means of intratesticular inoculation, starting with an emulsion of mouse spleen virus. The transmittal was completed the second or third day of the inoculation, at the moment when the testicular lesion was at its height. However, the testicular virus of the guinea pig was never of any great strength.

By using the intracerebral manner, Rivers and Berry (3) obtained the development of a characteristic disease with nearly all the inoculated animals. Very high fever, constant, reaches its maximum at the end of the first week after the inoculation. During this stage of fever, the animals refuse to eat losing weight and subject to ataxy or convulsive crisis. However, generally the animals are rapidly cured and are completely healthy two weeks after the inoculation. The psittacosis virus has indefinite propagation possiblities by means of this passing from brain to brain in the guinea pig. This passage in series in no way alters the virulence of the strain for parakeets and mice.

Fortner and Pfaffenberg (4) were able to start a fatal disease between the fifth and ninth days with strong dosage of virus introduced through the trachea or the peritoneal. They were able to locate the virus in organs. With small doses they caused chronic pulmonary lesions, without a virus present.

6. Rabbits.

Rivers and Berry (5), (6), Gordon (7) have proven the receptiveness of rabbit to the psittacosis virus.

Gordon working with this animal type caused a high tempo through intracutaneous inoculation, the tempo reached its maximum of inflammation from 40 to 60 hours after injection, remaining swollen during two to three days, then vanished, without ever turning vesicule.

Following intracerebral inoculation, with anrbmuln aviary strain passed through mice, Gordon observed in certain cases, an acute disease accompanied by paralysis, convulsions, often ending in death of the animal from the second to the fourth day. They only reached negative results with human virus.

Rivers and Berry described the disease given to the rabbit through intracerebral inoculation as an illness evolving towards a cure and transmissable in series. The virulence of this rabbits brain strain, is completely conserved through the parakeet and the mouse.

Following inoculation in the tracheal of the mouse virus, these authors (1) observed signs of fever and infection. A few animals died. During the autopsy the lungs presented pneumonia; the lung cultures remained bacteriologically sterile.

More recently Fortner and Pfaffenberg (2)were not able to infect the rabbit by muscular means. They caused pulmonary lesions by means of the trachea, but the animal remained outwardly in good health.

7. Monkeys.

Macaques monkeys of the type rhesus macacus inoculated by intratracheal or through the nostrils, with mouse virus, developed a bacteria free pneumonia. This pneumonia resembles if not completely similar to that observed in man. (Rivers and Berry 3).

Intracerebral inoculation of the virus causes meningo-encephalitis which is not fatal in the macacus, it is accompanied by no pulmonary manifestations.
                                        is possible
Transmission in series/with monkeys both by means of the trachea, or intra- cerebrally . However the virulence of these strains seems to get lost in both the case of the monkey and the mouse.

The intramuscular inoculation of the virus has not effect. However, it one takes pains to repeat the injection, one may notice mat neutralizing antibodies in the serum, and the animals inoculated in this way later resist to inoculation trhough the trachea. (See later chapter dealing with immunity).

Chapter V

Pathological Anatomy

1. Anatomo-Pathologic Lesions

In Man.
              of   who
At autopsy/the subjects/died from psittacosis one always finds lesions of inflammatory state of the lungs which is characteristic. The pulmonary lesions do not resemble in effect those which you find ih pneumonia or usual bronchial pneumonias. They are more like those found in influenza cases (Oberndorfer (1)).

In general, one finds pneumonia accompanied by muco-purulente bronchitis (Turnbull, Oberndorfer, Eogler). However Siegmund (2) and Giese (3) observed pulmonary lesions without alterations of the bronchi.

If in numerous cases, incision proves that there is pus in the bronchi (Obern...rfer) and if during the microscopic examination one finds under flammatory bronchiectasis at the level of the .... of the bronchi, it would seem we must consider the pulmonary lesions as not being resultant from their alteration of the bronchioli (Wilson 4). This muco purulente bronchitis with the presence of pneumo-bacteria must be considered as a terminal and independent complication of pneumonia (Tubnbull 5).

The characteristic lesion is therefore constituted by a vascular hemorrhaging pneumonia desquamitive, complication by a pulmonary thrombose and non microbial.

Microscopically it is presented itself under the aspect of red depatisation foci. ~~Xxxxxxxxxxxxxxxxxxxxxxx~~ During the ~~xxxxxxxxxxx~~ histological examination the epithelial of the alveoli is the seige of proliferation and marked desquamation. The cavities ~~xxxxx~~ are filled with an exudate which is first serous then fibrous with leucocytic infiltrations. However, in general the inflammatory cells are not numerous, these fibrino deposits being extremely rich in hematites. Moreover, one may notice an evermore marked thrombosis of the capillaries and the pulmonary arteries. Turbull considered this progressive development of thrombosis as the last step in inflammation.

Other writers (Oberndorfer, Giese) have described/hepatisation foci with
grey
fibrinous stroma and filled with leucocytes.

The plourous filtrates are not important. However one may note in numerous cases fibrinous deposits on the superfice and slight hemmorhaging

Other than the pleuro-pulmonary lesions a certain degree of digestion and of nervous edemia may sometimes exist. Herderschee (1)discovered a case of hemmorhagic pachymeningitis. However, moreoften the nervous system is normal. Sprunt and Corry (2) had noted small hemorrhages near the blood vessels and diagnosed them a cerbral purpura, in relation to the seriousness of the disease, but they do not have specific character.

On the other hand, microscopic examination of the spleen shows infiltration of large cells. The kidneys and the liver present a parenchmatic., hemmorhagic lesions exist mainly in the grand ~~xxxxxxxxx~~ abdominal tract (Tunbull, Herderschee, Oberndorfer).

In the Monkey.

It is interesting to compare the pulmonary lesions in human psittacosis htal cases and in those observed among experimentally diseased monkeys.

Rivers and Berry(3) were able to discover the way of extension and of formation of pulmonary lesions by sacrificing monkeys at specific intervals, after inoculation through the trachea or intranasally. Pneumonia sets in near the large bronchi, in the area of the helium and seem to spread towards the periphery, along the alveolar walls. Resolution follows the opposite path. During this evolution one may find a particular combination of ~~pathologenbic~~ varied pathological processess vascular tightening, desquamation of the alveolar epithelium, serous oozing, fibrin deposits, hemmorhages, necrosis of the alveolar walls, widening of the alveoles by polynucleurs and mononucleurs.

The meningitis encephalitis observed in the monkey, after intracerebral inoculation, is principally characterized by a mononucleur reaction at the level of the meninges.

Psittacides.

The lesions observed in the parrot and parrakeet are mainly localized in the spleen and the liver. The organs are hypertrophied and often small grayish nodules are found on their surface.

According to Mohs(1) one must consider any dead parrot with a spleen larger than 3.5 mm and less than 8 to 12 mm which is hypertrophy caused by simple infections as being suspect of having had psittacosis. When this split is from 5 to 8 mm the parrots have died of psittacosis. The histologic changes in the spleen are often unimportant; that they can also completely destroy the normal architecture, while the reticulum remains intact.

The characteristic lesion of psittacosis in parrots is found in the area of the liver: the microscopic examination shows numerous zones of necosy of the hepatic cells, irregularly distributed zones, but more numerous at the periphery. It is a question of the degenerative process during which the cell cytoplasm because acidophil and granulated and is retracted, while the center is charged with chromatin, and takes on a pyknotic aspect and often disappears. Then come leucocytes, mostly mononucleur, which infiltrate the lesion and may observe more than lecitary and fibrin deposits. A prolific amount of hepatic cells forms around the necrosed foci. At all levels a great amount of Kupffer cells which are tumorfied exist, vacuolized, full of yellowish pigment. The biliary canals are dialated and sometimes filled with mononucleurs in the necrotic zones. The vessels are not changed in a constant way, however they can be thrombotic, especially in the cases of small vessels.

Spread in the entire hepatic parenchyme, healthy or necrosed, one may find cells filled with elementary corpuscles about which we will speak later.

Beyond the splenic and hepatic alterations there are in general, in the area of the digestive tube, an inflammatory state and sometimes lesions of hemmorhagid enteritis. Peritonitis is often found. Levinthal(1), Elkeles(2) have also described, a pericarditis and pulmonary lesions of the pneumonic type at various degrees. This pulmonary ailment was also found by Sacquepee and Terrabouc(1); but it is very rare: Bedson, Rivers have never met up with it.

In the mouse.

After intraperitoneal inoculation, one finds an abundant peritoneal oozing among these animals, it is slimy containing fibrin flakes. The elementry corpus cles may be brought out on smears from this oozing(see furthur). The liver

is large, friable, of yellow color(Chamois), full of infarct. In cases of acute evolution, it presents under microscopical examination, infiltrations of fat, and degeneration of the hepatic cells, leaving necrose areas, infiltrated with polynucleurs and monocytes. In cases of slow evolution, the hepatic cells are nearly normal and one observes only mononucleur masses.

The spleen is swollen, very red and friable. The histological lesions consist of necrose zones, with leucocyte infiltrations, in the pulp and lymph follicles.

Mice infected intracerebrally, may present lesions of the same type, however these are less evident and less constant, in the area of the liver (20%) and the spleen(40%). Mainly, one finds a menengito encephalitis charactorized by an exudate composed of mono and polynucleur cells.

It should be noted, as of now, that the elementary corpuscles may be found easily in both incisions of organs andsmears from the exudates.

Characteristic lesions may be noticed in the area of the lungs of mice inoculated nasally. They have especially been studied by G. Hornus(1), whose description we borrow.

The lungs of mice which have died of pulmonary psittacosis may present various microscopic aspects. The lung often seems normal mainly of mice which have died procociously; only microscopic examination allows one to separate the elementary bodies and histological lesions. At other times it appears assa homogeneous block of pulmonary condensation. But most often, hepatization of a grayish pink color is observed, at times filling only a section of a lobe, at others filling the entire lobe. It is indeed exceptional that no large regions of the lungs of completely normal aspects are left. Lastly, and this mainly in cases of diseases that evolved very slowly(8 to 10 days), which may be noted after inoculation of dilutions, the lung is of nearly normal aspect, only seeded here and there with small grayish transluscent grains.

You should notice, that whatever the macroscopic aspect, the pulmonary edema is always of importance.

From a histopathological point of view, psittacotic pneumonia in the mouse is essentially characterized by a monocytic alveolitis, to which are added in variable degrees edematous and congestive lesions. The congestion which is more or less important, is noted mainly when illness is papidly evolved, Pulmonary edema is always of importance. The monocytic alveolitis is always clear when the lesions have evolved rapidly, in 3 to 4 days. But even in this case, and even moreso if the pneumonia is of the slowly evolving type, the polynucleurs which always exist in these lesions, are numerous. Necrotic foci appear at the same time.

The abundance of elementary bodies in the area of alveolitic foci is variable. They are sometimes rare; at other times on the other hand their abundance is extreme and the great majority of cells is filled with corpuscles. Pracytic cells are always mononucleur elements.

Affection of the bronchi is inconstant: they sometimes contain a varying number of cellular elements, monocytes and polynucleurs. These leucocytic masses parellely with the more or less important lesions of the bronchic epithelium.

G. Hornus discovered normally in all sections of pulmonary psittacosis, elementary bodies in the cells of the bronchic epithelium. The presence of these bodies does not necessarily imply deep lesions in these cells.

The experiments of G. Hornus were picked up by Rudd and Burnet (2) who observed that by using dilutions they limited the infecting power obtained from pulmonary lesions in foci. This foci may easily be counted clinical which provide a method of of finding the firus.

In the rabbit.

Except for light meningo-encephalitis which is common for all the infected animals (Rivers and Berry), the principal differences exist in lesions of the liver, found in 10% of the cases in which consist of degeneration of foci, of necrosis and and infarctus.

II. ELEMENTARY CORPUSCLES .

The publication by Bedson, Western and Levy Simpson, which demonstrates that the etiological agent does not belong to the class of filtrable virus, Levinthal (1), Lillie (2) and Coles (1) announced nearly simultaneously that they had observed on probably   colored preparations of the virulent material, very small formations at the limit of visability, mostly intracellular, were also found in a free state in the preparation. Lévinthal considered them as small bacteria of a similar nature as that of bacteria tularense and calls them "Microbacterium multiforms psittacosis". Lillie offered to call them "Rickettsia psittaci", and Coles, Corpuscule X. Whatever the denomination used these authors consider it the causal agent of the disease.

They are small round or slightly oval corpuscles resembling very little shells and appear in the smear either isolated or in pairs or in irregular groupings. Bacilli forms have been described by Coles, Lillie, Bedson and Western, but were not found later. It would therefore appear that these corpuscles are typically round or slightly oval. Their dimension varies from 0.25 microns to 0.45 microns with an average of 0.25 microns to 0.30 microns.

In sections and preparation made with impressions of the organs the corpuscles generally appear on the inside of reticulo-endothelial cells which sometimes are completely filled with them. However, G. Hornus (1) noticed on slides of pulmonary psittacosis from mouse, numerous of the elementary bodies in the light of the bronchi either in the interior of monocytes or free, extra cellular. They seem to make their way into the bronchial canal either by splitting of parasite of cells and dispersion of elementary bodies, or through desquamation of the epithelial cell.

Numerous extracellular forms may be found on the smears after a secondary washing of the cells.

In using Giemsa's method, after differentiation in the orange G. tannin solution, the elementary corpuscles take on a deep red color. They may also be colored with loeffler's blue and any polychrome blue. Rivers utes the coloration of the rickettsia modified by Castaneda.

Lepine and Sautter (2) were able to bring into evidence the presence of

thymonucleic acids in the elementary bodies of psittacosis by using the reaction of Feulgen, on slides from the lungs of mice infected intranasally.

Are these corpuscles really of the agents of the disease?

The fact that one was able to bring into evidence these formations in the virulent material derived from various sources supports this hypotheses: in the parrots, Levinthal and Rivers discovered it in the pericardic oozing; Rivers, in the liver, in man Coles discovered it in the blood; Lillie, in the pulmonary alveoli. In the mouse, in the blood and the spleen (Coles, Rivers), in the liver (Rivers), in the peritoneal exudate, in the mningo exudate, (Rivers), and in the alveoli of the lungs, in the cells of the alvolic epithelium and in the cells of the bronchic epithelium (Hornus). Rivers, however, noted that he did not always find these corpuscles in the virulent material in the origin of the parakeets and infected mice. He has also never been able to discover them in the pulmonary experimental lesions of the monkeys. Bedson and Western and also Elkeles and Barros had already noted the negative factor, in what concerns the aviary or human material. Bedson however (3) regularly discovered them in the spleen of infected mice and guinea pigs. However, the examination of a great number of mice suggests that the quantities of the corpuscles varies parallelly to the virulence (4).

The impossibility forth these corpuscles in certain types of virulent materials is not a sufficn5 reason to dispell the hypothesis that they are the virus. This is what more exact experiments conducted by Bedson tend to prove.

Bedson, having centrifuged a virulent emulsion during two hours at 5000 rpm, brought back the material to its original volume by adding physiological water and noticed that the virulence of this emulsion, tried on the guinea pig skin, proved itself identical to the original suspension. On the other hand, the examination of the obtained material in a smear proved that the elementary corpuscles had been concentrated in a noticable manner. Purification of the virus fraction centrifugation gave identical results and the material after being washed twice, contained only elementary corpuscles.

Lastly, the washed corpuscles of this particularly agglutinated by serum from guinea pigs inoculated against psittacosis and fixed the complement in the presence of the same specific serum.

III. EVOLUTIVE CYCLE OF THE VIRUS OF PSITTACOSIS

Bedson and Bland (1, 2) and Bland and Canti (3) continued the morphological study of the virus, not only in the mouse spleen infected experimentally, but also in tissue cultures. They noted morphologic changes taking place at regular intervals.

The first forms visible with a microscope are apparently homogenous groups. They are soon replaced by colonies (or "Morulae") of corpuscles of more or less equal size and having a diameter of approximately 1 micron. These large shapes are later multiplied by means of division and of a series of successive divisions, their size diminishes progressively and reaches that of elementary corpuscles.

The regularity with which this morphologial takes place, makes the authors believe that there is evolutive cycle. The fact to be noted is that when the

elementary corpuscles reach a convenient cell (either in the animal, or in cellular cultures), they are soon and constantly replaced by forms of larger dimensions.

This type of evolutive cycle would explain the pleomorphic aspect which Levinthal was the first to describe.

CHAPTER VI

CHARACTERISTICS OF THE VIRUS

Filtration

One of the essential characteristics of the psittacosis virus is to ~~yackbrough~~ ~~filters~~ cross through the following filters: Berkefeld IV (~~xxxx~~ positive results Armstrong and McCoy (1), ~~Berkefeld~~ Elkeles, negative results Levinthal).

Berkefeld V (positive results with Levinthal, Krummiede, Elkeles, Barros).
Chamberland L1, L2 (Bedson; Pesch (2)).
Chamberland L3 (Sacquepee; Pesch).
Seitz E K (Pesch; Bedson).
Reichel D (Elkeles).

According to Elkeles and Barros the best results were with Berkefeld~~ooooo~~ V and Chamberland L1.

Lazarus and Meyer (4) obtained completely different results: during their ~~xtixxbxxthxxb~~ trials, the Berkefeld V and Chamberland L3 candled and the Seitz EK filters held the virus. The differences probably hinges on the fact that the infectant material used was taken from cultures on the chorio-allantoic membrane of the incubated chicken egg.

Filtration results in a lessening of the virus. On the other hand, Armstrong noticed a short incubation for the filtered material—a difference that was not found by Pesch. According to Meyer and Eddie (5), there might be period ~~thr~~ of disease during which the virus does not penetrate filtered candling. Levinthal (6) discovered that the psittacosis virus measures 0.22 microns to 0.33 microns by the ultrafiltration technique used by Elford. In using this method modified by Bauer and Huges, Lazarus and Meyer (7) obtained constant results that it was at the source of the used virus, which allowed them to contribute a dimension of 0.200 microns to 0.300 microns to the virulent particles.

Conservation.

Virulent organs kept in a 50% glycerine solution at $\neq 6°C$ in the refrigerator conserves their activity during three weeks at least. (Bedson and Western 1, Gordons 2) and even during 36 to 66 days)(Meyer and Eddie). However, tissues kept in glycerine for a few days might lose their virulence (Bedson and Western).

Another method of consists of placing the infectious material in a phosphate solution of pH 7.6 at 6°C. Under these conditons, they are tested on the guirea pig's back, the tissue still shows virulence after the 55 day.(Bedson).

Meyer and Eddie picked up Gordon's experiments with the dessication of virulent organs. In drying infected mouse spleens, on calcium chloride or on phosphoric anhydride and by keeping them at $\neq 4^\circ C$, they retained their virulence during 277 days. On the other hand, dry excrements kept at room temperature, kaxixxkkxixxxdxxxdxxxxx would lose their virulence in 24 hours; in the icebox in 4 days.

Lastly, Bedson and Western conserved certain strains, by congelation, fxx (freezing) and found them virulent after the 50th day.(4)

Dilution.

Blood and spleen dilutions from infected mice were proven virulent up to 1/100,000 in what concerns the spleen and 1/10,000 for the blood as studied by Gordon (5). By inoculating mice with their dilutions up to 1/100,000,000 and 1/1,000,000,000 Fortner and Pfaffenberg (6) did not kill all experimental animals; a certain number escaped.

Centrifugation.

We show above that Bedson (7) had been able to concentrate and purify the virus by means of fractionated centrifugation; a centrifigue at 5000 rpm for 2 hours, goes back to psittacosis corpuscles while a centrifuge at 2000 rpm for 10 minutes has not action.

Heat.

Experiments conducted by Gordon on the mouse helped him estimate that 55 C for 30 minutes is sufficient to inactivate mostxxxxxx parrot strains, but does not always kill the virus. On the other hand exposed at 80 - 100 C for 30 mintes seems to kill the virus completely.

Action of Antiseptics

Permanganate of potassium diluted 1:10,000 at laboratory temperature, does not always kill virulence. The virus may also resist through an additional solution of 0.5% of phenol, even when the antiseptic is allowed to act at $37^\circ C$ for 20 hours. (Gordon).

The virus seems to resist the addition of a small quantity of ether; however, it is quite changed if the quantity added is 10%; at 5% one does not observe this change.

Treated with small doses of formol (1 to 2:1000) the emulsion of infected mouse spleen xxxxxxxx conserves its antigenic power thxxxx and loses its virulence. The virus treated in this way may be heated without xfxxxxxx lose of immunizing power while the natural virus when heated both loses virulence and antigenic (Bedson).

Culture of Virus.

We have shown above that Bedson and Bland (1) and (2) and Bland and Canti(3), during their research with the virus psittacosis morphology had used tissue cultures.

Haagen and Crodel (4) cultivated psittacosis virus in the Maitland nodium.

A number of tests made by MacCallum (5) show that the presence of live cells is indespensable for multiplication of psittacosis virus.

However, it mainly the various media and tissues of the chicken egg in the state of development were used for virus cultures.

Chorio-Allantoic Membrane

Burnet and Rountree (6), Fortner and Pfaffenberg (7) were able to obtain the psittacosis virus culture on the chorio-allantoic membrane.

Thevirus did not reduce as evident lesions as the vaccinal virus. The counting method by the number of papules is not usable. According to Rountree and Burnet the membrane three days after inoculation is thickened by the edema and shows a variable degree of capacity. Numerous little white opaque foci are dispersed on this surface, from 0.25 to 1.0 mm in diameter. These foci are usually small, but sometimes widespread and thicker.

With the strains used by Burnet and Rountree the embryo survived the inoculations and the lesion disappeared three to four days later.

Lazarus and Meyer (1) continued with the study of psittacosis virus culture on chorio-allantoic membrane. They conserved a strain for 38 months and effectuated 425 passages. As a whole they confirmed the above given results. However, they point out how rare lesions in the foci are, the only results of the inoculation being edemateux thickening of the membrane. Moreover, the embryo is killed the second or third day when the virus fixed on the egg by transferre. This period might be lenghtened if dilutions are injected: five to six days (dilution 1%) and even 6 to 8 days (dilution 1:1000).

Burnet and Rountree stated that they had no difficulty in infecting the membrane with a source of the virus was parrot or mouse lesions. On the other hand, Lazarus and Meyer noted few failures with suspensions from mouse spleens.

Later, the inoculation of virulent material (liver or spleen from psittacotic pigeons) into the chorio-allantoic membrane helped Smadel, Well and Gregg (2) to isolate five strains of virus.

Amniotic cavity.

Burnet and Foley (3) inoculated psittacosis virus into amniotic cavity and found that it multiplied. The amniotic liquid reached a high virus content. Inoculation in heavy doses of a strain which had been repeatedly transferred, caused a certain numberoof fatal cases with the embryo, after the 4th day.

The vitellin membrane.

Yanamura and Meyer (4) were able to obtain a psittacosis virus culture in the vitellin membrane of an embryo of approximately six days. A great number of virus were found after three days of incubation as well in the yolk as in the membrane itself.

Allantoic Cavity

is
Williams (5), showed that it was possible to cultivate the psittacosis virus in the allantoic cavity and that the quantity of collected virus were greater than when using cultures in the vitellin membrane. These realts were confirmed by R. D. Francis and Gordon (6) and were carried to two further groups: atypical pneumonia SF strain and meningo-pneumonia SF.97 strain. It should be noted that during those same series of experiments, Williams fialed with the lymphogranuolomatic virus and R. D. F rancis and Gordon with the mouse pneumonia.

CHAPTER VII

WAYS OF VIRUS PENETRATION

We have seen above (experimental study) that the psittacosis virus may be inoculated with positive results, by many means, to the laboratory animals. Here we will only remind of the results to clear up the epidemiologic problem. First of all the positive results obtained by Rivers following digestive and nasal inoculation in the parrots. It is the intratracheal and intranasal methods used by this author with the monkey and followed by pneumonia. The inoculation in the mouth (G. Hormus).

This ease of penetrating of the virus in the mucous of the digestive tract (parrots) and the respiratory tract (parrots, monkeys and mice) contrast in fact with the harmlessness of the intramuscular inoculation in man and monkey (see further the active immunization attempts).

REPARTITION OF THE VIRUS IN THE ORGANS OF THE INFECTED ANIMALS AND MEANS OF ELIMINATION

In the infected parrot both naturally and experimentally, the virus has been found in the liver (Beson, Rivers) in the blood and the spleen, in the lungs and nasal secretions (Rivers). Bedson discovered it in the intestines while Meyer and Eddie were not isolate with irregularity. The excrements were proven to be virulent (Rivers, Meyer, and Eddie). It would therefore appear that the means of elimination of the parrots are nasal secretionsand urine. In human patients, the virus has been isolated during inspection in the blood serum, and pleural secretions. (Bedson). Meyer and Eddie did not find it in the blood after the fourth day of illness. In using the white mouse as the control animal, Rivers proved the existance of the virus in the sputum taken between the 3rd and 9th day. Meyer and Eddie found the virus up to the 27th day, but insist on the fact that its immision is irregular and the examination should be repeated. The lung has been proven constantly virulent at autopsy; the spleen and the liver nearly always (Bedson, Meyer, and Eddie).

We have noted above (experimental study) that the repartition of the virus depends on the type of inoculation among laboratory animals experimentally infected.
under
With the help of this data, it is easy to state/what conditions human contagion takes place.

The most frequent is the contagion of bird to man. It is often made by direct contact. Or by indirect contact, by nasal mucus, excrements which are thrown into the air by birds and can be carried by air current. Man is infected by the respiratory

way and ~~xxxxxxxxxxpxxxxbxpxxkitx~~ a very short contact with virulent material
may be enough for infection to take place (1).

Contamination through diseased/ø human subjects are rare, but can be explained
by the virulence of the sputum.

CHAPTER VIII

CLASSIFICATION OF PSITTACOSIS AND ORNITHOSIS VIRUS

Remember that morphological and biological resemblances have allowed us to
group the psittacosis and ornithosisvirusthe following virus in a homogenous group:
The venereal lymphogranulomatosis virus of Nicolas and Favre disease.
The atypical human pneumonia virus (Strain S.F.)
The Meningo-pneumonic virus.
The cat pneumonia virus.
The mouse neumonia virus.
to which certain authors add the trachoma virus and that of blennorrhagia.

One may find all the details of resemblance and the means of diagnosis in
the article by J. C. Levaditi, dealing with the immunological and diagnostic
methods of the Nicolas and Favre disease, in the chapters dealing with the study
of reaction of the deviation of the complement and the reaction of neutralization.

Chapter IX

Immunity in Psittacosis
Natural immunity in man

During the epidemiological study we insisted on the rarity of psittacosis
among young children. Thus during the epidemic of 192901930, in England out of
104 cases studied, only 4 were under 10 years of age, and a critical examination
of these cases, made by Sturdy and Scott(1) led to doubt the ~~xxxxitude~~ exactness
of the diagnostic. It is also not exceptional, that during family epidemics,
young children do not catch the disease. And on the other hand, living under the
same conditions as their parents, one would suppose that they too had been in
contact with the virus.

These facts ~~xxxxxxxxxxxxxxxxxxxxx~~ which we cannot explain, allow
to suppose with a certain degree of certitude that a natural immunity towards
psittacosis exits in the child.

Immunity resuting from a first attack

Epidemiological studies have allowed to establish another fact: the low
number of cases among persons dealing with commerce of the birds. This relative
absence according to Sturdyeand Scott, might only be in appearance, but if it
is to be admitted, it could be explained that existence of a first attack,
accompanied by more orless grave clinical manifestations, and giving a certain
degree of immunity.

A case of relapse, has occurred in contradiction to this hypotheses; it is
a case observed by Wenkkebach(2) during a 2nd attack: sick once in 1930, he was
infected 6 years later in a laboratory, following manipulations of virulent material.

The lack of documents in human medicine does not allow any conclusion.

It is easier to reach conclusions when observing what happens among animals.

A certain degree of immunity following a first attack may be noted among cured psittacides.

Bedson and Western(1), when experimenting with parrots and zebra parrokeets, noticed that certain subjects resisted to inoculation of a controlled virulent material. Invaariably it concerned adult cases. Thus xhxxx one was carried to the hypotheses that it concerned birds immune from a previous natural infection. Following these observations, the authors used young animals, so as to avoid new failures.

A more precise experiment allowed Bedson and Western to prove the existence of a certain degree of immunity among parrakeets cured after first experimental infection; 8 of these birds were given a test inoculation;foùk showed nok symptoms, 3 were slightly sick, only one died.

Meyer(2) has shown that in many commercial bird enterprises, approximately 50% of the young parrots and approximately 10% of the adults have psittacosis virus in the spleen, the liver, and the kidneys(the virus being brought into evidence by inoculation in the mouse). The birds, in such a state of latent infection, resist to massive inoculation test doses, but one sacrificed, one may observe that they are all virus carriers, with a very small exception(from 2 to 10 %).

Similar facts have been observed among mice. Rivers and Berry(3) trying intraperitoneally 53 mice which had lived through a first inodulation, found only 5 who survived. Fortner and Pfaffenberg(4) trying a lot of mice which had resisted buccal inoculation, as well as high dilutions of virus (1/100,000,000 and 1/1,000,000,000). They proved to be immunized in a proportion of 20%.   x

On the other hand, Rivers and Schwentker(5) observed that monkeys cured of experimental psittacotic pneumonia, are less sensitive than new animals to a 2nd intratracheal virus inoculation. However this immunity is purely relative and kkkx if too great amount of virus is injected, the refractory state cannot be brought into evidence.

Neutralizing power of the serum

I. Serum from the convalescent

Bedson and Western (6) during their first experiments were not able to bring into evidence a neutralizing serum from the convalescent bird.

Rivers, Berry and Rhoads(1) were unable to get any neutralizing action in k using the mouse as the test animal. However when using intracerebral inoculation with the rabbit and taking as a criterion thermic elevation, they were able to find a very light neutralizing power, however so low that it kk was very difficult to demonstrate. In the same way, Sacqueree and Ferrabouc(2) while using the parrakeet for test inoculations, were unable to get definite results.

Later, Rivers and Schwenker (3) based their experirent on the proportion of dead mice and they used the average lenght of disease and used decreasing doses of virus; by this means, they were able to bring into evidence a certain number

of neutralizing antibodies in the serum, and a certain number of cases of psittacosis cured more or less recently(1 month to $3\frac{1}{2}$ years). However the inconstance of results in man, does not allow one to use the neutralization test for retrospective diagnosis of psittacosis.

On the other hand in the monkey, those authors were able to locate antibodies in the serum of animals killed of psittacotic experimental pneumonia from 39 days to 157 days. The difference in results could probably be explained by the time passed between the curing and seeking of antibodies.

2 Serum of hyperimmunized animals.

Having succeeded in immunizing mice by means of inactivated virus by means of the addition of formol(see below), Bedson and Western(4) noted that the serum from these mice was capable of neutralizing the virus. First to bring this neutralizing power into evidence, they inoculated the mixture virus-serum into the guinea pig by the intracutaneous means. The various virus dilutions in contact with the serum gave no cutaneous reactions. Bedson continued these experiments in the guinea pig(5). He intraperitoneally located virus in 2 of these animals and took samples of the serum after 9 to 15 injections. By testing the neutralizing power on mice, intraperitoneally by means of intraperitoneal inoculation of the mixture of virus-serum, he noted a very feeble protective effect(2 cases out of 6 examined); while on the skin of the guinea pig, the neutralizing is constant for the virus dilutions higher than 10^{-2} power. Bedson supposed that the difference in the results obtained is mostly owed to the great sensitivity of the mouse; on the other hand, it is possible that the virus-antibody complex is separated when injected intraperitoneally.

After having been able to immunize mice by means of a formol vaccine(see below) Bedson(6), demonstated the presence of specific antibodies circulating in the blood of mice who had been immunized. These antibodies present in feeble concentrations were brought into evidence by intraperitoneal inoculation of mixtures of virus-immune serum to the mouse. The injection of apparently neutral mixtures caused the development of an inapparent persistant infection.

On the other hand, having realized the pathogenic power of intranasal inoculation in the mouse, Rudd and Burnet(1) found no reduction of the virus activity following its exposure to the action of an invitro immune serum.

In the same way, Lazarus and Meyer (3) by inoculation of the chorioallantoic membrane of the incubated hens egg, were not able to demonstrate the presence of immune antibodies.

Agglutinating power of the immune serums

The washed elementary corpuscles are agglutinated by antipsittacosis serums from immunized guinea pigs(Bedson(2)). The reaction is extremely specific: An anti-spleen- mouse serum has not action on the corpuscles.

Lazarus and Meyer(3) studied the agglutinating power of serums from hyperimmunized animals (rabbits, guinea pigs, monkeys) and one human subject cured of natural infection. They noted that the agglutination of the elementary bodies by immune serum was specific and supposed that there existed 2 types of agglutinating: the one thermostable and the other thermolabile. On the other hand, a common antigenic factor was found present both in the elementary vaccine bodies

and in the psittacosis bodies. xxxxixxixxxingxxxxxxxxfxxxxxxxxxxxxxxxxxxx

Precipitating power of the immune serums

Preciptinogens were sought by Lazurus and Moyer (4) in the filtrates of tissues infected with Psittacosis(in fact, the chorio- allontoic membrane of the chicken embryo), but the results were not absolutely conclusive.

Fixation of the complement

The first attempts by Bedson and Western indicated the possibility of a certain specific fixation, with the serum of convalesence. Bedson was able to obtain through new experiments (5) (6) , with a great regularity, positive results, in utilizing serums from human psittacosis patients who had been cured (11 positive cases out of 12 examined). The reaction is always negative with control serums, exept when utilizes the Wassermann positive serums; these in fact, canstantly gave positive results, both with antipsittacotic antigen (spleen of infected mouse), and with control antigens, (nomral mouse spleen and ectromelic mouse spleens).

In psittacosis subjects, the reaction was found positive, after the 20th day of the illness and until the 5th week. It is probable that the reaction does not last long during the convalesence.

Moreover, Pedson insists that the fixing power of the complement of the serum is of a very low order and irdxxxdxxdxx that the examination must be conducted with rigor if one is to reach correct conclusions.

The results obtained by the same author with hyper-immunized guinea pig serum, are much more precise. This serum as a matterof fact, contains a high percentage of sensibility.

The difference existing between the neutralizing power of the anti psittacotic immune serums and their power of fixing the complement, according to Pedson, is probably due to the fact that the virus contains more than one antigen, and that it therefore, the serum contains more than one antibody.

In effect it would appear that the psittacosis virus contains 2 antigens, one resistant to boiling temperature, the other papidly destroyed at the same temperature(Bedson(1)). Each of these antigens determines the production of a specific antibody. The presence of these 2 antibodies and their reaction to their respective antigens were demonstrated invitro by the fixation of the complement. However the relationship between the 2 antibodies giving the fixation of the complement and the neutralizing power of the serum are not known.

Attempts at active immunization
Non attenuated virus.

Rivers and Schwentker(2) wondered, kpxxxixxg whether by using a different inoculation channel from that which causes the disease, they might confer a certain degree of immunity to subjects treated in this manner.

They injected psittacosis virus by intramuscular means into monkeys("Macacua rhesus) and noticed no disease symptoms, inxxxnnerxx neither in general, nor pulmonary or nervous. The injections were conducted at one week intervals in progressive doses of 0.1 cc, el15x 0.15 cc and one cc of virus. From 52 to 71 days after this

last injection, the animals were tested by means of intrtracheal virus inoculation and reacted in no way, while a non-vaccinated control animal, developed wide-spread pneumonia. Furthurmore, the serum of the vaccinated monkeys contained neutralizing antibodies.

Rivers and Schwentker applied the method to man: they take 6 intramuscular don attenuated virus injections, with succesive dilutions of 1/1000, 1/500, 1/200, 1/100, 1/10, During and after this series of injections, theyl observed no important sign of sickness and were able to control the appearence of neutralizing substances in the serum.

Attenuated virus.

A first experiment conducted by Bedson and Western (1) indicated that the virus of Psittacosis inactivated by means of formal is able to provoke the development of a certain immunity in mice: 3 out of 5 animals resisted the test inoculation. Later, Bedson(2) uses 3 types of vaccines: formalated, formalated and heated, heated. The virus inactivated by small quantities of formal may provoke a high degree of immunity. In the same manner, that formalated-heated virus possesses certain immunizing powers. On the other hand, the pure heated virus losses its virulence and its antigenic power.

Levinthal(3) obtained the same results with the formalated vaccine. He was also able to immunize mice with virus inactivated by the photodynamic method of Perdrau and Todd(methylene blue and radiationsx).

Rudd and Burnet(4) were not able to increase the mouse resistance to infaxk intranasal infection, by means of formalated vaccines. However, this vaccine produces ant important rise in resistance of the animals to intraperitoneal infection.

Formalated vaccines were prepared by Yanamura and Meyer(5), starting with tissue cultures and administered intraperitoneally. They proved effective in mice during test inoculations, administered in the same manner.

Wagner, Meiklejohn, Kingsland and Häckish(6) prepared vaccines based on infected vitelline membranes. These vaccines protected mice in 75% proportion, against intrperitoneal tests of 10,000 to 1,000,000 LD_{50} and provided complete protection against small doses of from 1 to 10 MLD intracerebrally or through the respiratory tract. The technique of vaccine preparation employed a lyphilization for the extraction formalate of ether. Such vaccines were extremely rich in elementary bodies. The authors noticed that the vaccine content of complement fixing antigen seemed to have not relation with its immunizing power.

Despite the important variations observed in the individual susceptibility of psittacides, Meyer, Eddie, and Yanamura(1) were able to administer an improtant degree of immunity to Melopsittacus undulatus and to Munia oryzivora, by the administration of formalated vaccine deprived of infectious powers, based on tissue cultures. HOwever, this immunity is incomplete; thus if the test inoculation is greater than 100 doses deathly to mice, Munia oryzivora is not protected. These facts demonstrate the difficulty of immixing active immunization of birds against psittacosis or ornithosis.

Passive Immunization Attempts

Passive immunization attempts were made by Rivers and Berry (2). They used

the serum of 10 human convalescents and that of a rabbit which had been inoculated intracerebrally. Mice were given 0.5 cc of serum, from 4 to 24 hours before the administration of 0.5 cc of virus intraperitoneally. All the animals died just as quickly as the controls treated with human serum or with normal rabbit serum.

Thalheimeir(3) has noted observing 2 human cases of psittacosis treated by injection of convalescent serum . While having evolved towards better health, they are not absolutely demonstrative, considering the late circumstance under which serotherapy was practiced.

ChapterX
Chemotherapeutic and Antibiotic Agents

Mauer(1), after chemotheropeutic tests, reported on the activity of trypaflavine on mouse psittacosis.

Rudd and Burnet(2) attempted to treat experimental mouse infection by means of sulphonamides: they were unaable to bring any action into evidence. These results were fully confirmed by Bedson (3) who discovered that the psittacosis virus is very rarely affected by sulphonamides.

Penicillin

The effectiveness of penicillin in experimental psittacisis of mice was demonstrated by Heilman and Herrell(4). The doses used were of 1000 units per mouse, divided into 5 doses, during every 24 hours for 5 days.

Parker and Diefendorf(5) studied the effects of penicillin on the multiplication of various virus in the Rivers-Li medium and in incubated Chicken eggs. Negative results were registered with equine encephalomyelitis virus, with the vaccine and the St. Louis encephalitis.

On the other hand, the scientist observed a negative action of penicillin on the development of psittacosis and meningito pneumonic virus uhder these conditions.

Bedson and May(6) confirmed these results; but underlined the fact that the quantity of penicillin needed to treat experimental psittacosis in mice is considerable: a minimum of 800 units per day was in fact found necessary.

Compared action of Sulphonamides and Penicillin

Early and Morgan(7) experimented with 6BC strain. They found that the sodium salt of sulfadiazine as also penicillin were effective agents in the treatment of psittacosis infections given intravenously or intraperitoneally to mice: under these conditions, the treated animals(orally) all survived. However, when using the intracerebral channel of virus inoculation, or the respiratory channel, the results obtained with sulfadiazine were superior to those of penicillin: mice inoculated nasally survived with disulfadiazine treatment; inoculated intracerebrally, they died 4 to 8 days after the controls.

The action of penicillin and of sulfadiazine on various strains of psittacosis and on a strain of ornithosis was studied by Meiklejohn, Wagner, and Beveridge(1) on the experimental infection of an incubated hens egg(inoculation in the allantoic liquid or the vitelline membrane of the embryo). Immediately injected, than 48 hours and 96 hours after the inoculation of the virus, penicillin showed up the inoculated embryos death with every strain under consideration; strains Borg, SP, Gleason and strain T-207 of pigeon ornithosis. On the other hand, sulfadiazine was only active

against 2 psittacosis strains. It only gave negative results in the cases of embyyos infected with psittacosis strains Borg and SF, and with that of Ornithhosis.

The action of the 2 agents was then studied in mice by Wiseman, Meiklejohn, Lackman, Wagner and Beveridge(2). For this study, the virus inoculation channel and the doses injected varied according to the pathogenic character of the strain (intravenous channels for the Gleason and Ornithosis strains; intraperitoneal for the Borg strain; intracerebral for the SF strain). Penicillin was injected subcutaneously or administered orally, which was also the form of administration of sulfadiazine.

As a whole, the results conformed to those obtained on the hen embryo. Penicillin has very definite therapeutic action on mice infected by the psittacosis Gleason and Borg strains and by pigeon ornithosis; It has only a light action on infections by means of the SF strain. Sulfadiazine, with no action inanimals affected by the Borg and Sf strains, and in those affected by pigeon ornithosis, is extremely active in the cases of the Gleason and 6BC strains, which confirms the research done By Early and Morgan noted above.

For an understanding fifx of the experimental results obtained through diverse medical agents, one must therefore carefully not e the virus strain used in the test.

It is interesting to note, that, in cases where mice treated with penicillin or sulfadi azine survive, the virus may be found in the surviving animals organs (Heilman and Herrel, Bedsonand May, Early and Morgan).

Certain experiments conducted by Early and Morgan(1) also demonstrat that the mode of action of sulfonamides and antibiotics is not simple.

Psittacosis virus(strain 6BC) in culture on embryonic chicken tissue resists to the action of striptomycin. Moreover, when it is not actively multiplying, the virus may survive in the presence of the salt of sodium sulfadiazine. The resistance of the virus in these conditions(Which is opposed its inhibition by sulfadiazine in tissue cultures and in incubated hens eggs) caused Early and Morgan to use Sulfadiazine and Streptomycin as protective agents atainst a possible contamination, the virus count remaining at a constant rate, in the presence of these agents. One could therfore use this method to isolate psittacosis virus from contaminated material.

From a clinical point of view, Toomey and Lonrey (2) report having observed 5 patients with bronchial pneumonic psittacosis and treated with sulphonamides 9sulphapyridine and sulfathiazole). They noted no direct amelioration caused by this treatment. However, despite the seriousness of their state, none of the patients died.

Despite the small number of observations which we were able to study, it would seem that the use of penicillin cusses a favorable effect on the evolution of the human diseas; thus ina case treated a s of the 5th day of the disease with 100,000 daily units, for 8 days, while not responding spectacularly to the treatment, Turgassen(3) reports that the temperature dropped back to normal on approximatly the 10th day of disease. Flippin, Gaydosh and Fittopoldi(4), observed that penicillin therapy was followed by a definite clinical amelioration within 36 hours.